

# Interleukin-1 Blockade Attenuates Mediator Release and Dysregulation of the Hemostatic Mechanism During Human Sepsis

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**Objective:** To define the influence of interleukin-1 activity on coagulation and fibrinolytic system activation and the release of proinflammatory mediators in the early human response to severe infection.

**Study Design:** All patients with severe sepsis syndrome who were enrolled from two surgical centers that were participating in a randomized, double-blind, placebo controlled, multicenter, multinational trial of recombinant human interleukin-1 receptor antagonist in the treatment of sepsis syndrome.

**Population:** Twenty-six patients with sepsis syndrome received an intravenous loading dose of recombinant human interleukin-1 receptor antagonist (100 mg) or placebo followed by a continuous 72-hour infusion of recombinant human interleukin-1 receptor antagonist (1.0 [n=9] or 2.0 [n=8] mg/kg per hour) or placebo (n=9).

**Outcome Measure:** Responses up to 72 hours after initiation of treatment.

**Results:** Plasma levels of the anaphylatoxin C3a and

thrombin-antithrombin III complexes were reduced in the high-dose recombinant human interleukin-1 receptor antagonist treatment group after 72 hours ( $P<.05$ ). Similarly, parameters of fibrinolysis, tissue-type plasminogen activator, and plasminogen activator inhibitor type 1 but not plasmin- $\alpha_2$ -antiplasmin complexes, were also significantly reduced ( $P<.05$ ) after 72 hours of treatment with a high dose of recombinant human interleukin-1 receptor antagonist. Neutrophil elastase- $\alpha_1$ -antitrypsin complexes and phospholipase  $A_2$  levels were also significantly reduced in the high-dose recombinant human interleukin-1 receptor antagonist treatment group after 72 hours.

**Conclusions:** The results confirm that activation of the coagulation and fibrinolytic systems and release of soluble inflammatory mediators are consistently observed in patients with severe sepsis syndrome. Interleukin-1 activity contributes to activation of these processes as documented by the reduction in surrogate activation markers during recombinant human interleukin-1 receptor antagonist treatment.

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**S**EPSIS SYNDROME, characterized by hypotension, vascular collapse, and multiple organ failure, is a significant cause of morbidity and mortality in hospitalized patients and continues to be an important health care problem. It is generally accepted that this syndrome is caused by an excessive release and activation of endogenous inflammatory mediators in response to the infecting microorganisms. Cytokines, particularly interleukin-1 (IL-1) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), have been implicated as important mediators in the initiation of this inflammatory host response.<sup>1</sup> In accordance herewith, levels of TNF- $\alpha$  and IL-1 $\beta$  are elevated during experimental endotoxemia<sup>2,3</sup> as well as during septic shock in baboons<sup>4</sup> and humans.<sup>3,5</sup> The intravenous administration of tumor necrosis factor in

animals results in shock, multiple organ failure, and death,<sup>6,7</sup> whereas infusion of recombinant interleukin-1 in rabbits<sup>8,9</sup> and primates<sup>10</sup> induces similar cardiovascular effects. Moreover, TNF and IL-1 may synergize in inducing hypotension, tissue injury, and death,<sup>8,11</sup> as well as in triggering the release of IL-6<sup>12,13</sup> and presumably IL-8.<sup>14-16</sup> Their overlapping biological effects may be due, at least in part, to stimulation of the production of TNF by IL-1 and vice versa,<sup>17,18</sup> a situation that very likely occurs in septic shock.<sup>12</sup>

A number of in vitro and in vivo studies have suggested that TNF and IL-1

See Methods on next page.

## METHODS

### PATIENTS

Twenty-six patients were entered in this study. The selection of patients is outlined below. The patients were part of a larger multicenter phase III randomized, double-blind, placebo-controlled study to evaluate the efficacy of interleukin-1 receptor antagonist in reducing 28-day all-cause mortality rate in patients with severe infection. In the present study, all patients from the Free University Hospital (Amsterdam, the Netherlands) and the New York (NY) Hospital who were included in the larger multicenter trial entered this substudy. All patients gave written informed consent, and the protocols were approved by the institutional review boards of each participating hospital.

Patients were randomized, as described previously,<sup>30</sup> to one of the following treatment groups: interleukin-1 receptor antagonist, 1.0 mg/kg per hour (n=9); interleukin-1 receptor antagonist, 2.0 mg/kg per hour (n=8); or placebo (vehicle; n=9). These groups are designated below as the low-dose, the high-dose, and the placebo groups, respectively. The treatment regimen in both interleukin-1 receptor antagonist treatment groups was initiated with a 1-minute intravenous bolus of 100 mg of interleukin-1 receptor antagonist in 10 mL of 0.9% sodium chloride solution or an equivalent volume of vehicle in 10 mL of 0.9% sodium chloride solution in the placebo group. Thereafter, interleukin-1 receptor antagonist was administered by continuous intravenous infusion for 72 hours in 0.9% sodium chloride solution infusion bags at an infusion rate of 12 mL per hour. All patients received standard intensive care support, including intravenous administration of fluids, cardiovascular and respiratory support, and administration of appropriate antimicrobial agents, and they could undergo surgery when indicated. All patients started with the assigned treatment within 24 hours after the diagnosis of sepsis syndrome was made. Prior to the start of the interleukin-1 receptor antagonist or placebo infusion, data were collected for calculation of the Acute Physiology and Chronic Health Evaluation (APACHE II) score according to the methods of Knaus et al<sup>31</sup> to demonstrate that severity of illness was similar among treatment groups.

### SELECTION OF PATIENTS

Inclusion criteria of the multicenter trial were as follows: (1) clinical evidence to support a presumptive diagnosis of sepsis syndrome of a presumed infectious origin; (2) fever or hypothermia (core temperature greater than or equal to 38°C or less than or equal to 36°C); (3) tachycardia ( $\geq 90$  beats per minute); (4) tachypnea ( $\geq 20$  breaths per minute) or requirement of mechanical ventilation; and (5) either hypotension (systolic blood pressure of 90 mm Hg or less, mean arterial pressure of 70 mm Hg or less, a decrease in systolic blood pressure of 40 mm Hg or greater, the need for vasopressors [except dopamine administered at a dos-

age of less than 5.0  $\mu\text{g/kg}$  per minute] to stabilize blood pressure in the presence of adequate fluid resuscitation), or any two or more of the following signs of organ dysfunction and/or inadequate perfusion: (a) acute deterioration in mental status (in the absence of sedative hypnotic drugs or other therapeutic agents with central nervous system depressant effects); (b) arterial hypoxemia ( $\text{PaO}_2$  of 75 mm Hg or less or a  $\text{PaO}_2$ -fraction of inspired oxygen ratio of 250 or less); (c) metabolic acidosis ( $\text{pH} \leq 7.30$  or base deficit of 5.0 mEq/L or greater) or increased plasma lactic acid concentration; (d) oliguria (urine output of 0.5 mL/kg per hour or less); (e) coagulation abnormalities (prothrombin time or partial thromboplastin time of greater than or equal to 1.2 times the upper limit of normal); (f) thrombocytopenia ( $\leq 100 \times 10^9/\text{L}$  or a decrease of 50% or more); (g) cardiac index greater than 4.0 L/min per square meter with systemic vascular resistance less than 800 dynes- $\text{sec-cm}^2$  in the presence of adequate fluid resuscitation. All criteria had to have been met in 24 hours or less prior to treatment.

Patients meeting any of the following criteria were not eligible to participate: age of 18 years or younger; pregnancy; weight greater than 100 kg; evidence of nonseptic cardiogenic shock or a source of uncontrollable severe blood loss; severe, preexisting, parenchymal liver disease with clinically significant portal hypertension; anuria ( $\leq 50$  mL of urine output per day); experiencing rejection of solid organ or bone marrow transplantation; current immunocompromised condition (including but not limited to corticosteroid administration [ $\geq 1.0$  mg/kg per day of prednisone or equivalent], chemotherapy, or radiation) or a disease sufficiently advanced to suppress resistance to infection (including but not limited to leukemia, lymphoma, acquired immunodeficiency syndrome, and known human immunodeficiency virus seropositivity); full-thickness thermal or chemical burn involving 30% or more of total body surface area; receipt of an investigational new drug within the previous 30 days; non-resuscitation agreement; or presence of an irreversible, rapidly fatal underlying disease or injury.

### DRUGS

Human recombinant interleukin-1 receptor antagonist (Anakinra, Synergen Inc, Boulder, Colo) was provided in a sterile solution (vehicle) of pH 6.5 that contained sodium chloride, trisodium citrate, disodium citrate, citric acid, edetate disodium, polysorbate 80, and sterile water. As a placebo, the vehicle was used.

### BLOOD COLLECTION

For the present study, blood samples were only taken during the time that treatment with interleukin-1 receptor antagonist or placebo was given (72 hours). Thus, although patients were followed up for 28 days, the evaluation period for the present study was considered to be 72 hours. Blood samples were obtained through the patient's arte-

exert their influence during sepsis via the induction of other mediators such as IL-6 and IL-8, arachidonic acid metabolites, and platelet activating factor. These proinflammatory cytokines also induce activation of coagulation and fibrinolysis pathways and induce activation of neutrophils.<sup>8,12-16,19-25</sup> It has yet to be determined to what

extent TNF- $\alpha$  and IL-1 contribute to the pathogenesis of the sepsis syndrome in humans, either directly or via induction of other inflammatory mediator species.

Recently, a recombinant human antagonist of IL-1 receptors became available for use in clinical studies.<sup>26,27</sup> This interleukin-1 receptor antagonist binds to

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rial line and were collected in 10-mL siliconized tubes that contained 3.8% wt/vol sodium citrate or 150 U of heparin sodium. The blood samples were taken shortly before and at 4, 12, 24, 48, and 72 hours after the start of the treatment regimen. After collection, they were immediately stored in ice to prevent activation of the complement system. Plasma was obtained by centrifugation of blood for 15 minutes at 1300g. All plasma samples were stored in aliquots at  $-70^{\circ}\text{C}$  until tested.

## ASSAYS

Plasma TNF- $\alpha$  and IL-1 $\beta$  levels were measured by standard enzyme-linked immunosorbent assay (ELISA) techniques, as reported elsewhere.<sup>32,33</sup> The sensitivity of these assays is 34 and 8 pg/mL, respectively.

The concentration of IL-6 in plasma was quantified with an ELISA modified from that described in detail before.<sup>34</sup> Briefly, purified monoclonal anti-interleukin-6 antibody (mAb CLB-IL6/16) was used as a capture antibody, and biotinylated sheep antibodies in combination with streptavidin-polymerized horseradish peroxidase conjugate (CLB Department of Immune Reagents, Amsterdam) were used to detect bound IL-6. Results were expressed as picograms per milliliter by reference to a standard consisting of recombinant human IL-6.<sup>35</sup> The lower detection limit of this assay was 5 pg of IL-6 per milliliter of plasma.

Interleukin-8 was measured with a sandwich ELISA modified from that described previously<sup>36</sup>; monoclonal anti-interleukin-8 antibody (mAb CLB-IL8/1) and biotinylated affinity-purified sheep anti-interleukin-8 antibodies were used as capture and detecting antibodies, respectively. Polymerized horseradish peroxidase conjugated to streptavidin was used to quantify bound biotinylated antibodies. Results were compared with those obtained with dilutions of recombinant human IL-8 and expressed as picograms per milliliter. The lower detection limit of this assay was 5 pg/mL.

The anaphylatoxin C3a in plasma was measured by a radioimmunoassay as previously described.<sup>37</sup>

Thrombin-antithrombin III (TAT) complex levels were determined with a novel ELISA. In this assay, monoclonal antibody TR3 against human thrombin and prothrombin was used to catch TAT complexes from samples to be tested. Bound complexes were detected using biotinylated monoclonal antibody ATIII-0 (CLB Department of Immune Reagents) in combination with streptavidin-polymerized horseradish peroxidase. Results of this ELISA were expressed as nanograms per milliliter by reference to a standard consisting of pooled human serum. (The amount of TAT complexes in this in-house standard was assessed using a commercial ELISA for TAT complexes [Behringwerke AG, Marburg, Germany]). The lower limit of detection of this assay was 1 to 2 ng of TAT complexes per milliliter. Normal values (ie, obtained from a panel of normal donors) were less than 4 ng/mL. Comparison of levels in plasma samples from patients with varying levels of TAT com-

plexes (from normal to greater than 1000 ng/mL) obtained with this novel assay for TAT complexes with those measured by a commercial assay (Behringwerke) yielded an excellent correlation ( $r=.99$ ,  $n=23$ ).

Tissue-type plasminogen activator (t-PA) concentrations in plasma were measured with a previously described sandwich ELISA,<sup>38</sup> and plasmin- $\alpha_2$ -antiplasmin (PAP) complex levels were measured with a previously described radioimmunoassay.<sup>39</sup>

Plasminogen activator inhibitor type 1 (PAI-1) levels were assessed with an ELISA that had been modified from a sandwich-type radioimmunoassay as described in detail before.<sup>40</sup> In short, monoclonal anti-PAI-1 antibody (mAb CLB-2C8) was used as the coating antibody and biotinylated polyclonal rabbit anti-PAI-1 antibodies as the conjugate. Results were expressed as nanograms per milliliter by reference to a standard curve of human PAI-1. This assay had a lower detection limit of 5 ng/mL.

Neutrophil elastase- $\alpha_1$ -antitrypsin complexes were determined with a radioimmunoassay as described in detail before.<sup>41</sup>

Phospholipase A<sub>2</sub> concentrations in plasma were determined with an ELISA that had been modified from that of Smith et al.<sup>42</sup> Monoclonal antibodies against human secretory PLA<sub>2</sub> type II (sPLA<sub>2</sub>) were used as the coating and catching antibodies. Results were compared with those obtained with cultured medium from Hep G2 cells stimulated with human interleukin-6.<sup>40</sup> The amount of PLA<sub>2</sub> in this cultured medium was assessed by comparison with purified recombinant human sPLA<sub>2</sub>. The lower limit of detection in this assay was 1 ng/mL; the mean  $\pm$  SEM normal value as assessed in 19 healthy volunteers was  $20 \pm 7$  ng/mL (range, 9 to 30 ng/mL).

All of the above assays exhibited interassay variation coefficients of less than 15%, as was estimated from the variation of dose-response curves obtained on at least 3 different days over a 3-month period. To minimize interassay variation, all samples were tested within one assay procedure.

## STATISTICAL METHODS

The data are expressed as means  $\pm$  SEMs. Factorial analysis of variance (ANOVA) was used for comparison of APACHE III scores and demographic data between groups. An ANOVA for repeated measures was used to assess significant changes in variables in the course of the observation period. The nonparametric Mann-Whitney *U* test was used to determine the significance of the differences between groups. The Wilcoxon rank sum test was used to assess the differences within groups between baseline levels and those at subsequent time points. For all tests, a two-tailed *P* value less than .05 was considered statistically significant. The analysis was performed using a commercial statistical package (Stat-View, Abacus Concepts Inc, Berkeley, Calif) on a Macintosh computer (Apple Computer Inc, Cupertino, Calif).

both types of IL-1 receptors with the same affinity as IL-1 but does not induce signal transduction. Interleukin-1 receptor antagonist has been useful in evaluating the influence of IL-1 on secondary mediator systems and physiologic measures of experimental sepsis. In a primate model of lethal septic shock, administration of interleukin-1 re-

ceptor antagonist improved survival and hemodynamic performance and reduced IL-6 levels without affecting circulating TNF concentrations.<sup>28</sup> Moreover, administration of interleukin-1 receptor antagonist abrogated aggregation of neutrophils and lung injury in endotoxin-induced<sup>9</sup> and *Escherichia coli*-induced shock<sup>29</sup> in rabbits.

In the present study, we tested the hypothesis that IL-1 may contribute to the activation of other mediator systems such as the complement, coagulation, and fibrinolytic cascades in human sepsis. We also sought to evaluate the role of IL-1 on neutrophil activation and the release of both arachidonic acid metabolites, IL-6 and IL-8. For this purpose, we prospectively analyzed the course of the plasma levels of these mediators during selective inhibition of IL-1 in patients with sepsis syndrome who were enrolled in a phase III study to evaluate the efficacy and safety of interleukin-1 receptor antagonist. The clinical results of this trial have been reported previously.<sup>30</sup> Our results indicate that clinical administration over a period of 72 hours of a large molar excess of interleukin-1 receptor antagonist relative to interleukin-1 that is sufficient to block nearly all the IL-1 receptors attenuated activation of the complement, coagulation, and fibrinolytic systems. Furthermore, such intervention reduced the levels of IL-6, neutrophil elastase, and phospholipase A<sub>2</sub> (PLA<sub>2</sub>) in patients with sepsis, suggesting that IL-1 activity contributes to the appearance of these inflammatory mediators during human infection.

## RESULTS

### PATIENTS

The three patient groups accrued from these two institutions were balanced for APACHE III scores at study entry (**Table 1**) ( $P=.86$ ). In addition, when the groups were compared for distribution of age and gender, no significant differences were observed. The diagnoses on admission to the intensive care units (all patients undergoing

surgery) were the following: intra-abdominal infection ( $n=6$ ); bowel infarction ( $n=3$ ); pancreatitis ( $n=3$ ); pneumonia ( $n=3$ ); gastrointestinal tract perforation and peritonitis ( $n=2$ ); multiple trauma, including intra-abdominal lesions ( $n=2$ ); gastrointestinal tract bleeding ( $n=2$ ); abdominal aortic aneurysm, ruptured or infected ( $n=2$ ); leg amputation ( $n=1$ ); necrotizing fascitis and cellulitis ( $n=1$ ); pelvic abscess ( $n=1$ ); cholangitis ( $n=1$ ); systemic infection of unknown origin ( $n=1$ ); and line sepsis ( $n=1$ ). The three treatment regimens were randomly distributed within the more frequent disease categories.

### PLASMA LEVELS OF TNF- $\alpha$ AND IL-1 $\beta$

Plasma levels of TNF- $\alpha$  were undetectable except in a few patients who had detectable TNF- $\alpha$  concentrations at only one time point. Similarly, IL-1 $\beta$  was only randomly detected in the plasma among treatment groups, and such levels were near the detection limit of this assay. Because of the low incidence of detectable TNF- $\alpha$  and IL-1 $\beta$  levels, neither calculations of means nor statistical analysis of the above data was performed.

### EFFECT OF IL-1 RECEPTOR BLOCKADE ON PLASMA LEVELS OF IL-6 AND IL-8

The baseline levels ( $t=0$  hours) of IL-6 (**Table 2**) were not statistically significantly different between groups ( $P=.6$ ). In the placebo group, IL-6 levels remained unchanged during the observation period, whereas administration of interleukin-1 receptor antagonist at either 1.0 or 2.0 mg/kg per hour resulted in a progressive decrease of IL-6 plasma levels over 72 hours. Interleukin-6 levels in the low-dose and high-dose groups were below the levels of the placebo group from  $t=24$  hours and  $t=48$  hours onward, respectively (data not shown), and remained lower until the end of the treatment period (**Table 2**). Differences between the groups were not statistically significant at any time point. When the course of IL-6 within each group was considered, both interleukin-1 receptor antagonist treatment groups but not the placebo group showed a significant decrease from  $t=4$  hours onward compared with their respective baseline values. In the high-dose group, this decrease was highly significant at  $t=4$  hours,  $t=12$  hours, and  $t=24$  hours (**Table 3**).

Baseline levels of IL-8 were higher in the high-dose group than those of the low-dose and placebo groups, although this difference did not reach statistical signifi-

**Table 1. APACHE III Scores at Study Entry and Demographic Data**

| Treatment Group† | APACHE III Score‡ | Age, y     | Gender  |
|------------------|-------------------|------------|---------|
| Placebo          | 78.9 ± 5.0        | 63.5 ± 3.3 | 6 M/3 F |
| Low-dose         | 77.2 ± 5.9        | 69.4 ± 2.6 | 5 M/4 F |
| High-dose        | 72.1 ± 4.3        | 57.6 ± 7.3 | 5 M/3 F |

\*No significant differences were observed between groups, as determined by analysis of variance. APACHE III indicates Acute Physiology and Chronic Health Evaluation. Values are mean ± SEMs.

†Low-dose group was administered interleukin-1 receptor antagonist at a rate of 1 mg/kg per hour; high-dose group, 2 mg/kg per hour.

‡According to the methods of Knaus et al.<sup>31</sup>

**Table 2. IL-1 Receptor Blockade and Plasma Levels of IL-6 and IL-8**

| Treatment Group† | IL-6, pg/mL |           | IL-8, pg/mL |           |
|------------------|-------------|-----------|-------------|-----------|
|                  | $t=0$ h     | $t=72$ h  | $t=0$ h     | $t=72$ h  |
| Placebo          | 243 ± 57    | 276 ± 179 | 423 ± 245   | 492 ± 368 |
| Low-dose         | 190 ± 174   | 43 ± 29   | 673 ± 577   | 172 ± 46  |
| High-dose‡       | 133 ± 360   | 63 ± 37   | 894 ± 350   | 156 ± 66  |

\*Values are mean ± SEM plasma concentrations of interleukin-6 (IL-6) and IL-8 at study entry ( $t=0$ ) and after a 72-hour infusion period ( $t=72$ ). No significant differences were observed between groups.

†Low-dose group was administered interleukin-1 receptor antagonist at a rate of 1 mg/kg per hour; high-dose group, 2 mg/kg per hour.

‡Significant difference ( $P<.05$ ) within group between  $t=0$  and  $t=72$ .

Table 3. Differences ( $P$  Values) Within Groups Between Baseline Levels ( $t=0$ ) and Those of Subsequent Time Points\*

|                        | t=0 | t=12  | t=24  | t=48  | t=72  |
|------------------------|-----|-------|-------|-------|-------|
| <b>Placebo Group</b>   |     |       |       |       |       |
| IL-8                   |     |       |       |       |       |
| IL-6                   |     |       |       |       |       |
| C3a                    |     |       |       |       |       |
| ELAST- $\alpha_1$ -AT  |     |       |       |       |       |
| PAP                    |     | <.05† |       | <.05† | <.01† |
| TAT                    |     |       |       |       |       |
| t-PA                   |     |       |       |       |       |
| PAI-1                  |     |       |       |       |       |
| sPLA <sub>2</sub>      |     |       |       |       |       |
| <b>Low-Dose Group</b>  |     |       |       |       |       |
| IL-8                   |     | <.05† | <.05† | <.05† | <.05† |
| IL-6                   |     |       |       |       |       |
| C3a                    |     |       |       |       |       |
| ELAST- $\alpha_1$ -AT  |     |       |       |       |       |
| PAP                    |     | <.05† |       |       |       |
| TAT                    |     |       |       |       |       |
| t-PA                   |     | <.05† | <.05† |       | <.05† |
| PAI-1                  |     |       |       |       |       |
| sPLA <sub>2</sub>      |     |       |       | <.01† | <.05† |
| <b>High-Dose Group</b> |     |       |       |       |       |
| IL-8                   |     | <.01† | <.01† | <.05† | <.05† |
| IL-6                   |     | <.05† |       |       |       |
| C3a                    |     |       | <.05† |       | <.05† |
| ELAST- $\alpha_1$ -AT  |     |       |       |       | <.05† |
| PAP                    |     |       |       |       |       |
| TAT                    |     | <.01† | <.01† | <.05† | <.05† |
| t-PA                   |     |       | <.05† | <.05† | <.05† |
| PAI-1                  |     |       | <.01† | <.05† | <.05† |
| sPLA <sub>2</sub>      |     |       | <.05† | <.05† | <.05† |

\*Baseline levels ( $t=0$ ) are the values before the start of the infusion. Differences within groups were determined using the Wilcoxon rank sum test. The low-dose group was administered interleukin-1 receptor antagonist at a rate of 1 mg/kg per hour; high-dose group, 2 mg/kg per hour. IL indicates interleukin; ELAST- $\alpha_1$ -AT, neutrophil elastase- $\alpha_1$ -antitrypsin; PAP, plasmin- $\alpha_2$ -antiplasmin; TAT, thrombin-antithrombin III; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; sPLA<sub>2</sub>, secretory phospholipase A<sub>2</sub>; and ellipses, not significant.

†Significantly increased.  
‡Significantly decreased.

cance (high dose vs placebo,  $P=.6$ ; high dose vs low dose,  $P=.3$ ) (Table 2). Interleukin-8 levels in both interleukin-1 receptor antagonist treatment groups were below those of the placebo group from  $t=48$  hours onward and remained at these levels until the end of the treatment period (Table 2). However, there was no statistically significant difference between the interleukin-1 receptor antagonist treatment groups and the placebo group at any time point. In the placebo group, a modest decline in IL-8 level was observed at 12 to 24 hours, after which, values returned to baseline levels (data not shown). Compared with the placebo group, the decline in the low-dose group was protracted for at least 48 hours, but this difference did not reach statistical significance (Table 3). In contrast, a significant decrease in IL-8 levels compared with baseline values was found in the high-dose group at  $t=4$  hours and  $t=24$  hours (Table 3).

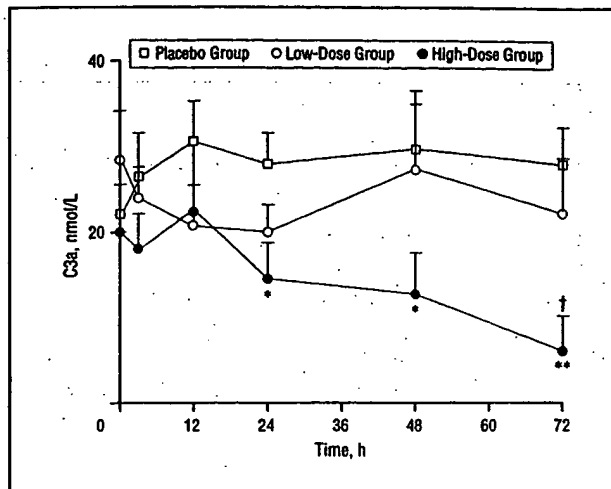


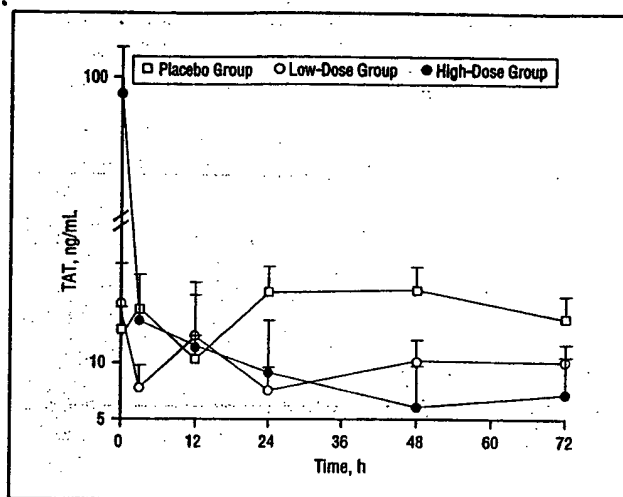
Figure 1. Interleukin-1 receptor blockade and complement activation: mean  $\pm$  SEM plasma concentrations of C3a during a 72-hour infusion period in placebo and low-dose (1 mg/kg per hour) and high-dose (2 mg/kg per hour) interleukin-1 receptor antagonist groups. Significant differences between the placebo group and the high-dose interleukin-1 receptor antagonist group are indicated by an asterisk ( $P<.05$ ) or double asterisks ( $P<.01$ ), and those between low-dose and high-dose interleukin-1 receptor antagonist groups are indicated by a dagger ( $P<.05$ ) (Mann-Whitney U test).

## EFFECT OF IL-1 RECEPTOR BLOCKADE ON COMPLEMENT ACTIVATION

Plasma levels of C3a were assessed to estimate the extent of complement activation in the treatment groups. Baseline levels of the placebo, low-dose, and high-dose groups were comparable ( $22.0 \pm 3.6$ ,  $28.1 \pm 5.0$ , and  $22.9 \pm 5.5$  nmol/L, respectively). In the placebo group, a modest increase in circulating C3a levels was observed during the 72-hour period after the infusion had started (Figure 1). This increase was significant compared with baseline values at  $t=12$  hours ( $P<.05$ ; Table 3). In the low-dose group, levels of C3a gradually declined and tended to be less than those in the placebo group from  $t=4$  hours onward. However, neither this difference nor the decrease compared with baseline values was statistically significant. In contrast, the C3a levels in the high-dose group declined over time, achieving a significant difference compared with baseline values at  $t=24$  hours and  $t=72$  hours (Table 3). Compared with the placebo group, levels of C3a were significantly lower in the high-dose group at  $t=24$  hours,  $t=48$  hours,  $t=72$  hours ( $P<.05$ ,  $P<.05$ , and  $P<.01$ , respectively; Figure 1). At  $t=72$  hours, the high-dose group showed a significant decrease compared with the low-dose group ( $P<.05$ ), which suggests a dose-dependent effect of interleukin-1 receptor antagonist on diminishing circulating C3a levels.

## IL-1 RECEPTOR BLOCKADE AND THE COAGULATION SYSTEM

Levels of plasma TAT were measured to assess the activation of the coagulation system. Baseline values were only moderately elevated in the placebo and low-dose groups, whereas a higher baseline value was observed in the high-dose group. At  $t=0$  hours, three of eight patients in the high-dose group demonstrated higher lev-



**Figure 2.** Interleukin-1 receptor blockade and the coagulation system: mean  $\pm$  SEM plasma concentrations of thrombin-antithrombin III (TAT) complexes during a 72-hour infusion period in placebo and low-dose (1 mg/kg per hour) and high-dose (2 mg/kg per hour) Interleukin-1 receptor antagonist groups.

els of TAT complexes when compared with patients in both other groups (**Figure 2**). However, the differences between baseline levels of the groups were not statistically significant, nor were the differences at subsequent time points. In the high-dose group, a highly significant decline from baseline values was noted at  $t=4$  hours and thereafter (Table 3), reaching TAT levels below those measured in the placebo group 48 hours after start of the infusion (Figure 2).

#### IL-1 RECEPTOR BLOCKADE AND THE FIBRINOLYTIC SYSTEM

Plasma levels of t-PA and its main inhibitor, PAI-1, and PAP complexes were measured to assess the effect of IL-1 receptor blockade on fibrinolytic system activation (**Figure 3**). Baseline levels of t-PA and PAP complexes in all three patient groups were comparable (Figure 3, top and bottom). In the placebo group, levels of t-PA did not change during the observation period but were only moderately elevated. Following infusion with interleukin-1 receptor antagonist, a gradual decline of t-PA levels was noted in the low-dose group, whereas the high-dose group showed a rapid decline that reached the detection limit of the assay within  $t=48$  hours (Table 3 and Figure 3, top).

Activation of fibrinolysis was observed in the placebo group, and PAP complex levels increased during the observation period (Figure 3, bottom). These levels were significantly increased above baseline values at  $t=12$  hours,  $t=48$  hours, and  $t=72$  hours ( $P<.05$ ,  $P<.05$ , and  $P<.01$ , respectively) (Table 3). Although an initial increase in PAP complex levels also was observed in both interleukin-1 receptor antagonist treatment groups, this increase did not continue during the 72-hour observation period, as was noted in the placebo group (Figure 3, bottom). However, no significant differences were noted at any time point between groups.

All three study groups demonstrated a decline in PAI-1 levels during the observation period (Figure 3, cen-

ter). In the placebo and low-dose groups, levels of PAI-1 gradually decreased from baseline values but never reached a statistically significant difference from baseline. In contrast, the high-dose group demonstrated a significant decline in PAI-1 levels that was noted within 24 hours and onward (Table 3). This decline coincided with that of the t-PA levels in this group. In the placebo group, levels of PAI-1 tended to decrease, whereas t-PA levels remained unchanged.

For all measured parameters of fibrinolysis, the levels tended to be lower in the high-dose interleukin-1 receptor antagonist group than in the low-dose group, which suggests a dose-dependent effect of interleukin-1 receptor antagonist on the reduction of fibrinolytic system activation.

#### EFFECT OF IL-1 RECEPTOR BLOCKADE ON NEUTROPHIL ACTIVATION

Neutrophil elastase- $\alpha_1$ -antitrypsin complexes were also measured to assess neutrophil activation in these patients. Levels of neutrophil elastase- $\alpha_1$ -antitrypsin gradually declined in both interleukin-1 receptor antagonist treatment groups, notably within 24 hours after the onset of the infusion. In the high-dose group, a significant decrease in neutrophil elastase- $\alpha_1$ -antitrypsin levels was observed at  $t=72$  hours compared with baseline values ( $P<.05$ ) (Table 3). By contrast, the levels of neutrophil elastase and antitrypsin remained unchanged in the placebo group (**Figure 4**).

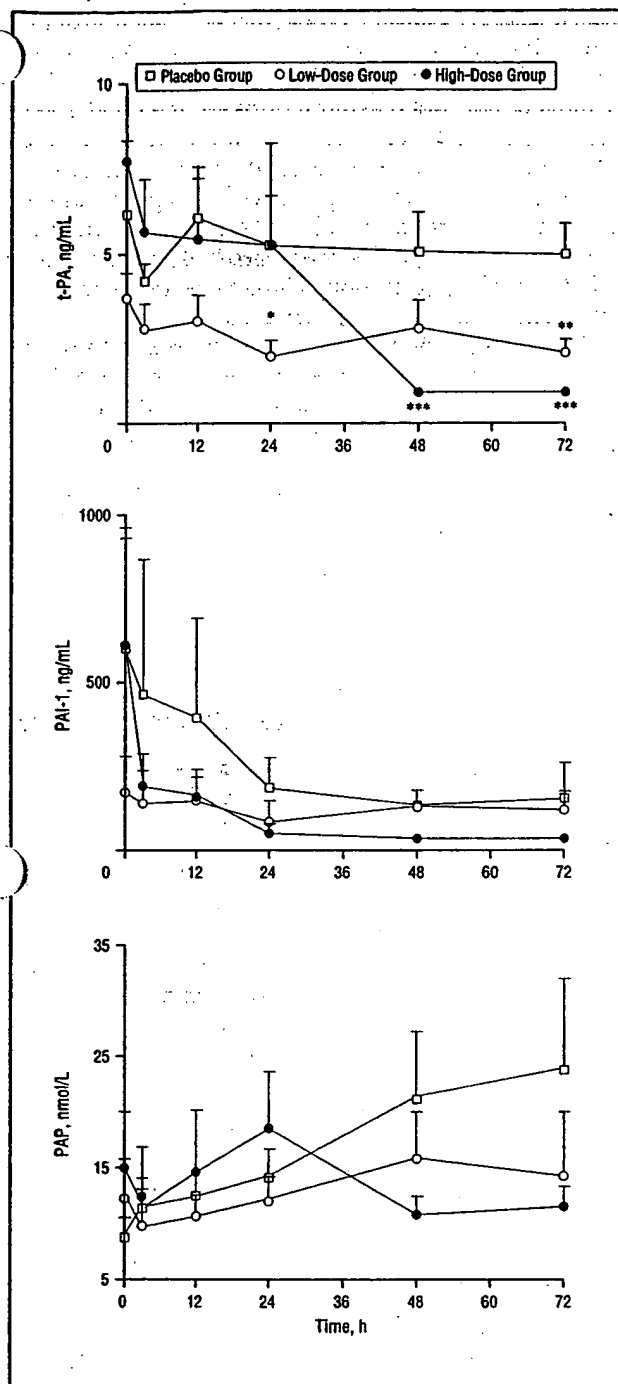
#### IL-1 RECEPTOR BLOCKADE AND PLASMA LEVELS OF PLA<sub>2</sub>

The activity of phospholipase A<sub>2</sub> is suggested to be the rate-limiting step in the biosynthesis of arachidonic acid metabolites.<sup>43</sup> Therefore, levels of sPLA<sub>2</sub> were measured to estimate the in vivo effect of IL-1 receptor blockade on the formation of proinflammatory lipid mediators. Baseline levels of sPLA<sub>2</sub> were comparable between all three groups (**Figure 5**). The levels of sPLA<sub>2</sub> remained unchanged during the 72-hour observation period in the placebo group, whereas those in the low-dose and high-dose groups declined significantly from baseline values from 24 hours onward (Table 3). In the high-dose group, the levels were significantly lower at two time points ( $P<.05$ ) when compared with those in the placebo group (Figure 5).

#### COMMENT

The cytokines TNF- $\alpha$  and IL-1 are presumed to initiate many aspects of the systemic inflammatory response via activation of various mediator systems. However, the relative contribution of TNF- $\alpha$  and IL-1 to this host response remains unclear. The relative role of IL-1 as a proximal signal in this process was investigated by serially evaluating plasma levels of purported IL-1-inducible inflammatory mediators in patients with sepsis syndrome during infusion of a low or high dose of interleukin-1 receptor antagonist. During such interleukin-1 receptor





**Figure 3.** Interleukin-1 receptor blockade and the fibrinolytic system: mean  $\pm$  SEM plasma concentrations of tissue-type plasminogen activator (t-PA) (top), plasminogen activator inhibitor type 1 (PAI-1) (center), and plasmin- $\alpha_2$ -antiplasmin (PAP) (bottom) complexes during a 72-hour infusion period in placebo and low-dose (1 mg/kg per hour) and high-dose (2 mg/kg per hour) interleukin-1 receptor antagonist groups. Significant differences between placebo and respective interleukin-1 receptor antagonist treatment groups are indicated by an asterisk ( $P < .05$ ), double asterisks ( $P < .005$ ), and triple asterisks ( $P < .001$ ) (Mann-Whitney U test).

antagonist treatment, levels of IL-6 and, to a lesser extent, IL-8 were significantly reduced compared with levels prior to intervention. The levels of such secondary mediators in the placebo group remained unchanged. It was similarly observed that measures of the complement, coagulation, and fibrinolytic system activation also

declined in interleukin-1 receptor antagonist-treated patients without a comparable reduction in the placebo group. Finally, levels of neutrophil elastase and soluble PLA<sub>2</sub> also decreased during the 72-hour treatment period, particularly in the high-dose interleukin-1 receptor antagonist treatment group.

Although a limited number of patients were studied, the levels of inflammatory mediators of the three patient groups were comparable at the time of study entry. Although the mean baseline levels in the high-dose group were higher than those in the low-dose or placebo groups for six of nine measured variables, further comparison of initial APACHE III scores as well as demographic data revealed an equal distribution of these variables among the three study groups.

Changes in secondary mediator levels over time were most prominent in the high-dose interleukin-1 receptor antagonist group. For those parameters in which a reduction in secondary molecules was noted, this generally occurred to a greater extent in the high-dose interleukin-1 receptor antagonist group, further suggesting a dose-dependent influence of interleukin-1 receptor antagonist treatment. This trend was further supported by the course of changes in inflammatory mediator levels following completion of the 72-hour interleukin-1 receptor antagonist treatment period. Plasma samples were also obtained 24 hours after the planned discontinuation of interleukin-1 receptor antagonist infusion in 16 patients. At this time point, several levels of inflammatory mediators increased in patients treated just prior with interleukin-1 receptor antagonist: IL-6 (five of nine patients), IL-8 (four of 10), C3a (five of nine), TAT (three of 10), and PAP (four of 10). This occurred despite the fact that a progressive decrease in the levels of these variables had been observed in all these patients in the preceding 48 to 72 hours. By contrast, none of the patients in the placebo group ( $n=5$ ) sampled at this time point demonstrated change in measured variables (data not shown).

The detection of circulating IL-1 $\beta$  in patients with sepsis has been noted in up to 37% of patients with sepsis,<sup>3,5,44,46</sup> and such results may be influenced by the assay methods.<sup>47</sup> A very low incidence of detectable IL-1 $\beta$  was observed in our study. This may be because we collected the initial blood sample just prior to the start of treatment (12 to 24 hours after admission to the intensive care unit). This may also explain why TNF- $\alpha$  was also seldom detected. In patients with sepsis, TNF- $\alpha$  is rapidly eliminated from the circulatory system.<sup>46</sup> The current study underscores the concept that a lack of detection of IL-1 $\beta$  in the plasma may not reflect activity at the tissue level.

Numerous studies have implicated a key role for IL-1 in the activation and release of various inflammatory mediators, either directly or via the induction of IL-6 and other cytokines.<sup>13,16,17,48</sup> In accord with the stimulating effect of exogenous interleukin-1 $\alpha$  on the release of IL-6 observed in nonhuman primates,<sup>10</sup> IL-1 receptor blockade attenuated circulating levels of IL-6 during human sepsis. In addition, plasma IL-6 responses to lethal *E coli* shock are significantly diminished by interleukin-1 receptor antagonist treatment in baboons.<sup>28</sup> Earlier phase

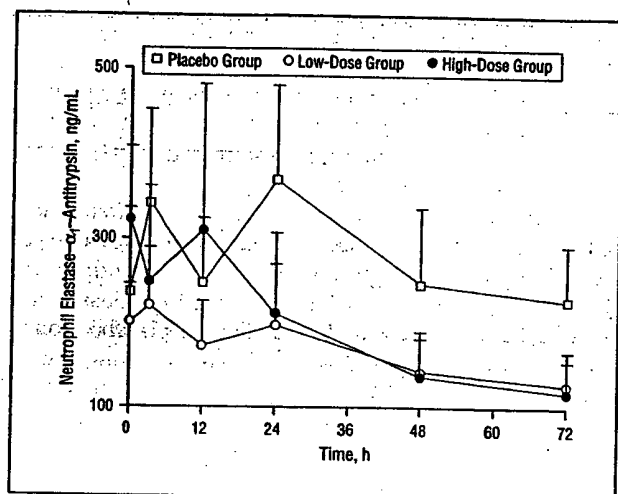


Figure 4. Interleukin-1 receptor blockade and neutrophil activation: mean  $\pm$  SEM plasma concentrations of neutrophil elastase- $\alpha_1$ -antitrypsin complexes during a 72-hour infusion period in placebo and low-dose (1 mg/kg per hour) and high-dose (2 mg/kg per hour) interleukin-1 receptor antagonist groups.

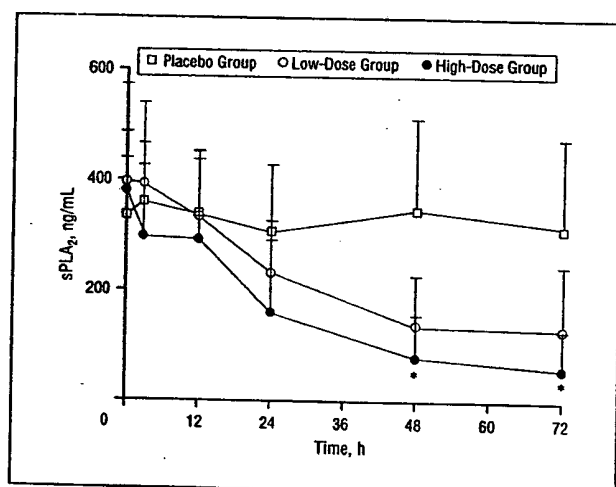


Figure 5. Interleukin-1 receptor blockade and phospholipase A<sub>2</sub>: mean  $\pm$  SEM plasma concentrations of secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) during a 72-hour infusion period in placebo and low-dose (1 mg/kg per hour) and high-dose (2 mg/kg per hour) interleukin-1 receptor antagonist groups. Significant differences between the placebo and high-dose interleukin-1 receptor antagonist treatment groups are indicated by an asterisk ( $P < .05$ ) (Mann-Whitney U test).

IL results also demonstrated a dose-proportional reduction of IL-6 levels during interleukin-1 receptor antagonist treatment.<sup>49</sup> This effect may well be of clinical importance because increased IL-6 levels have been found to predict a fatal outcome in patients with sepsis syndrome.<sup>44,50,51</sup> To what extent this reduction in IL-6 levels exerts a direct influence on other mediator systems remains to be determined.

Neutrophil activation and migration<sup>52</sup> is influenced by IL-8, and levels of this cytokine increase during intravenous administration of interleukin-1 $\alpha$  in primates<sup>14</sup> and are also elevated during human sepsis.<sup>36</sup> In accordance with the lack of significant reduction in IL-8 levels during interleukin-1 receptor antagonist treatment in the present study, Fischer et al<sup>28</sup> observed no effect of IL-1 receptor blockade on circulating levels of IL-8 in baboons with sep-

sis. No data are available on the role of IL-1 in the induction of IL-8 in patients with clinical sepsis, and our data provide no evidence to suggest a significant role for IL-1 in the appearance of IL-8 during sepsis. Interestingly, however, interleukin-1 receptor antagonist treatment did produce a decrease in levels of circulating neutrophil elastase (complexed to  $\alpha_1$ -antitrypsin), which suggests diminished activation and degranulation of neutrophils. In agreement herewith, in rabbits challenged with endotoxin or *E coli*, neutrophil aggregation in lung tissue was reduced by interleukin-1 receptor antagonist treatment.<sup>9,29</sup> In vitro studies have shown that interleukin-1 receptor antagonist can bind to type II IL-1 receptors on human neutrophils,<sup>53</sup> whereas the ex vivo experiments of Fasano et al<sup>25</sup> have demonstrated an increased expression of IL-1 receptors in patients with sepsis syndrome.<sup>25</sup> Thus, our results suggest that IL-1 is involved in neutrophil activation in patients with sepsis syndrome.

Complement activation during severe infection is generally considered to result from a direct interaction of complement proteins with bacteria. A complement activation mechanism induced by IL-1 has yet to be described. However, evidence for the existence of a cytokine-inducible pathway has been provided by evaluation of the complement system in patients with cancer who received immunotherapy with recombinant interleukin-2. In these patients, administration of recombinant interleukin-2 yielded a dose-dependent increase in plasma levels of C3a, indicating activation of the complement system.<sup>54</sup> This complement activation was presumed to be an indirect effect of recombinant interleukin-2 administration, probably via the induction of other cytokines, because in vitro recombinant interleukin-2 did not activate the complement system.<sup>54</sup> De Boer et al<sup>55</sup> found a biphasic activation pattern of complement activation in baboons challenged with live *E coli*, the second phase starting 4 to 6 hours after the challenge, at which time the infused microorganisms had been cleared from the circulatory system. In the present study, we observed a dose-dependent reduction in circulating C3a levels during interleukin-1 receptor antagonist infusion, which suggests that an endogenous activation mechanism of complement by IL-1 may occur in patients with severe infection. As neutrophilic proteinases are able to cleave complement factors,<sup>56</sup> the effect of IL-1 on the activation and degranulation of neutrophils may be significant in this respect. It is noteworthy that activation of the complement system, as reflected by elevated plasma levels of C3a and C4a, correlates with a fatal outcome in patients with sepsis.<sup>57</sup> Thus, reduction of complement activation via inhibition of IL-1 may be of clinical importance.

Disseminated intravascular coagulation is a major complication of sepsis and results from disturbances of the hemostatic balance of the vascular endothelium. Following endothelial damage, the coagulation system in plasma is activated. This system consists of two cascades, ie, the extrinsic or tissue factor pathway and the intrinsic pathway or contact system of coagulation. Activation of the coagulation system via either route can be quantified by assessment of plasma levels of thrombin complexed to its specific inhibitor, antithrombin III (TAT complexes). This coagulative response is accompanied by a biphasic change



in the fibrinolytic system involving initial stimulation and subsequent inhibition of plasminogen activation<sup>58,59</sup> as measured by plasma levels of plasminogen complexed to its inhibitor,  $\alpha_2$ -antiplasmin (PAP complexes). In both experimental endotoxemia and septic shock, activation of fibrinolysis is evident by an early increase in t-PA activity followed by a decline that is coincident with the appearance of the main inhibitor of t-PA (PAI-1).<sup>59,60</sup> A similar pattern has been demonstrated following a TNF challenge in healthy volunteers: the coagulative response is favored as the fibrinolytic response is inhibited after its initial increase.<sup>20,21</sup> Interleukin-1 receptor blockade markedly attenuated activation of coagulation in our patients, as was evident from the course of TAT complexes. These results are in agreement with in vitro data demonstrating that IL-1 enhances tissue factor expression on human endothelial cells,<sup>61</sup> thereby inducing activation of the extrinsic pathway of coagulation. Furthermore, cultured endothelial cells release PAI-1 in response to TNF and IL-1.<sup>23,62</sup>

The increase of PAI-1 levels in human sepsis is further documented by our data, which also suggest that IL-1 also stimulates activation of the fibrinolytic system by inducing the release of t-PA and the activation of plasminogen. Conversely, both TNF and IL-1 diminish the secretion of t-PA by cultured human endothelial cells.<sup>23,62</sup> Thus, the in vivo behavior of TNF in human volunteers as well as our data illustrate a discrepancy between in vitro results and the in vivo situation. Alternatively, thrombin potentially induces the release of t-PA and PAI-1.<sup>63</sup> As interleukin-1 receptor antagonist treatment induced decreased circulating TAT complex levels, the decreased release of t-PA and its inhibitor also may have been due to a diminished thrombin formation following interleukin-1 receptor antagonist infusion.

Arachidonic acid derivatives (eicosanoids), such as thromboxane A<sub>2</sub>, leukotrienes, and prostaglandins, have potent effects on platelets and neutrophils as well as alter vascular permeability and tissue blood flow.<sup>64</sup> The release of arachidonic acid from membrane phospholipids is ascribed largely to the hydrolytic action of PLA<sub>2</sub>.<sup>43</sup> Moreover, PLA<sub>2</sub> is a key regulatory enzyme for the production of platelet activating factor, which has also been implicated as an important mediator of sepsis and septic shock.<sup>19,65</sup> Elevated levels of secretory PLA<sub>2</sub> correlate to hypotension and pulmonary changes (adult respiratory distress syndrome) in patients with sepsis.<sup>66,67</sup> In vitro studies have demonstrated that TNF, IL-1, and IL-6 may induce secretion of PLA<sub>2</sub> by various cell types, such as hepatoma cells.<sup>68</sup> Redl et al<sup>69</sup> recently confirmed the role of TNF as a mediator of PLA<sub>2</sub> in baboons with sepsis. Our data further extend the in vivo role of cytokines toward the release of PLA<sub>2</sub> by demonstrating that IL-1 receptor blockade induced a dose-dependent decrease of PLA<sub>2</sub> levels.

Our results lead us to conclude that the activation and/or release of inflammatory mediators during severe infection is at least partly attributable to IL-1 activity. The current results further substantiate a pivotal role for this cytokine in the pathogenesis of a generalized host inflammatory response. The attenuation of coagulation, fibrinolytic and complement activation variables, levels of secretory PLA<sub>2</sub>, and, to a lesser extent, neutrophil elastase during interleukin-1 receptor antagonist treatment are consistent with the increase

in survival time in patients with disseminated intravascular coagulation or adult respiratory distress syndrome present at study entry, as observed in a previously reported multicenter phase III trial<sup>30</sup> in which these patients were accrued. Although this trial did not achieve a statistically significant reduction in the all-cause 28-day mortality rate in interleukin-1 receptor antagonist-treated patients, the current data demonstrate that interleukin-1 receptor antagonist treatment diminishes the appearance of several inflammatory variables and the alterations of coagulation and/or fibrinolysis pathways. These observations lend further support to the concept that IL-1 activity is of importance in the pathogenesis of the more severe manifestations of the systemic inflammatory response syndrome.

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Reprints not available.

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